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## 3, 3', 4, 4'-Tetrachloroazobenzene (TCAB)

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NTP Board of Scientific Counselors

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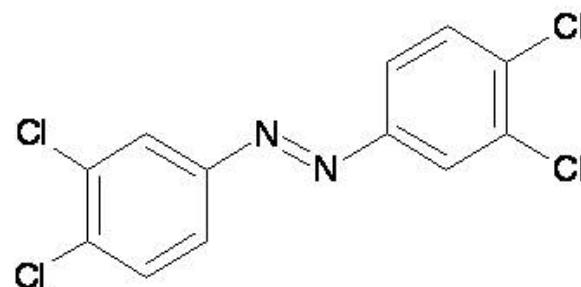


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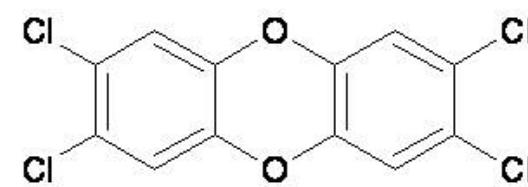
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## Rationale for Study

- Nominated by USEPA for toxicity testing based on:
  - Potential for human exposure
  - Structural similarity to TCDD
    - Binds/activates AhR resulting in dioxin-like effects
    - May account for significant amount of dioxin-like activity in the environment



TCAB



TCDD



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## Study Objectives and Design

- Characterize toxicity and carcinogenicity in rats and mice
- Compare the potential tumor outcome with TCDD
  - Evaluated as part of the NTP TEF program
  - Determine a relative potency factor for TCAB
  - Currently not included in the WHO TEF methodology
- Study design
  - Gavage route of administration
  - 3-month studies in F344/N rats and B6C3F1 mice
  - 3-month studies in Harlan Sprague-Dawley (HSD) rats
  - 2-year studies in HSD rats and B6C3F1 mice



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## Results of 3-Month Studies in Male and Female HSD Rats

- Doses of 0, 0.1, 0.3, 1, 3, 10, 30 and 100 mg/kg
- No effects on survival or body weight
- Regenerative anemia observed in males
- Decreased T<sub>4</sub> serum concentrations
  - No effect on T<sub>3</sub> or TSH
- Increased liver and lung P450 enzyme activities
- Dose dependent increase in TCAB tissue levels
  - Adipose>liver>lung>blood
  - Liver:Adipose ratios <0.015
    - Suggests no CYP1A2 binding in the liver



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## Results of 3-Month Studies in HSD Rats Cont'd

- **Changes in organ weights**
  - Increased liver, kidney and spleen weights in males
  - Increased liver and lung weights in females
  - Decreased thymus weights
- **Histopathology**
  - Hepatocellular hypertrophy
  - Bronchiolar metaplasia of the alveolar epithelium (Males)
  - Hematopoietic cell proliferation in the liver and spleen
  - Thymic atrophy
  - Severity generally minimal- not dose limiting



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## Summary of 3-Month Studies in HSD Rats

- Effects observed are typical of dioxin-like compounds
- Similar effects were observed in F344/N and HSD rat strains
- TCAB is six orders of magnitude less potent than TCDD based on CYP1A1 induction
- Doses of 0, 10, 30 and 100 mg/kg were selected for 2-year studies



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## Survival and Body Weights of 2-Year HSD Rats

	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
<b>Survival</b>				
Males	28	9	4	2
Females	25	30	19	17
<b>Body Weight (% Control)</b>				
Males	-	89	88	80
Females	-	105	87	80

N=50

Decreased survival in males due to lung tumors



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## Primary Neoplastic and Nonneoplastic Lesions

- Increased incidences of lesions in a number of tissues
  - Lung
  - Liver
  - Oral mucosa
  - Thyroid (Males)
  - Skin (Males)
  - Forestomach (Females)
  - Adrenal cortex (Females)

Nonneoplastic lesions observed in a number of additional tissues



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## Neoplastic Lesions in HSD Rats

Male	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
<b>Lung</b>				
Cystic keratinizing epithelioma	0**	14**	31**	37**
<b>Liver</b>				
Cholangiocarcinoma	0*	4*	4*	6**
<b>Oral Mucosa</b>				
Squamous cell carcinoma	1	5*	4	5*
Female	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
<b>Lung</b>				
Cystic keratinizing epithelioma	0**	6*	26**	39**
<b>Liver</b>				
Cholangiocarcinoma	1	1	1	3
<b>Oral Mucosa</b>				
Squamous cell carcinoma	0*	0	4	6*

N=49 or 50 animals; \* P≤ 0.05; \*\* P≤ 0.01



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## Neoplastic Lesions in HSD Rats

Male	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
<b>Thyroid Gland</b>				
Follicular cell adenoma	0*	3	4*	4*
<b>Skin</b>				
Malignant schwannoma	0*	0	1	3
Female	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
<b>Forestomach</b>				
Squamous cell papilloma or carcinoma (combined)	0*	1	0	4
<b>Adrenal Cortex</b>				
Adenoma	1	1	3	4

N=49 or 50 animals; \* P≤ 0.05



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## Conclusions in Male and Female HSD Rats

- *Clear evidence of carcinogenic activity* based on:
  - Cystic keratinizing epithelioma of the lung
  - Gingival squamous cell carcinoma of the oral mucosa
  - Cholangiocarcinoma of the liver (Males)
- Were also related to treatment:
  - Follicular cell adenoma of the thyroid gland (Males)
  - Cholangiocarcinoma of the liver (Females)
  - Squamous cell papilloma or carcinoma (combined) of the forestomach (Females)
- May have been related to treatment:
  - Malignant schwannoma (Males)
  - Adenoma of the adrenal cortex (Females)



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## Results of 3-Month Studies in B6C3F1 mice

- Doses of 0, 0.1, 1, 3, 10 and 30 mg/kg
  - High dose limited by thymus weights
- No effects on survival or body weight
- Organ weights
  - Increased liver and spleen weights
  - Decreased thymus weights (Males)
- Histopathology
  - Squamous hyperplasia in the forestomach epithelium
  - Hepatocellular hypertrophy (Males)
  - Hematopoietic cell proliferation in the spleen (Males)
  - Severity generally minimal- not dose limiting



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## Survival and Body Weights of 2-Year B6C3F1 Mice

	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
<b>Survival</b>				
Males	35	31	5	0
Females	35	30	32	20
<b>Body Weight (% Control)</b>				
Males	-	97	83	-
Females	-	104	93	90

N=50

Decreased survival in males due to urethral tumors (urinary obstruction) and in females due to urethral and skin tumors



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## Primary Neoplastic and Nonneoplastic Lesions

- Increased incidences of lesions in a number of tissues
  - Urethra
  - Lung
  - Forestomach
  - Skin- Females
  - Malignant lymphomas- Females
- Nonneoplastic lesions observed in a number of additional tissues



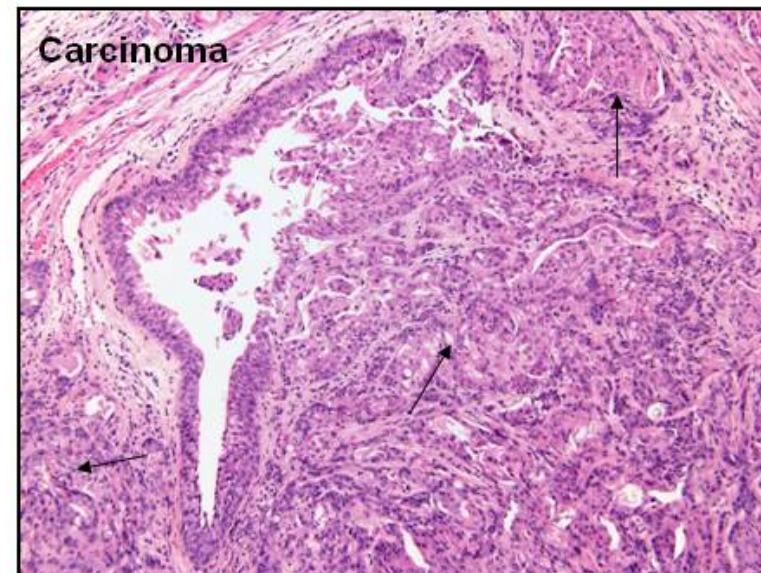
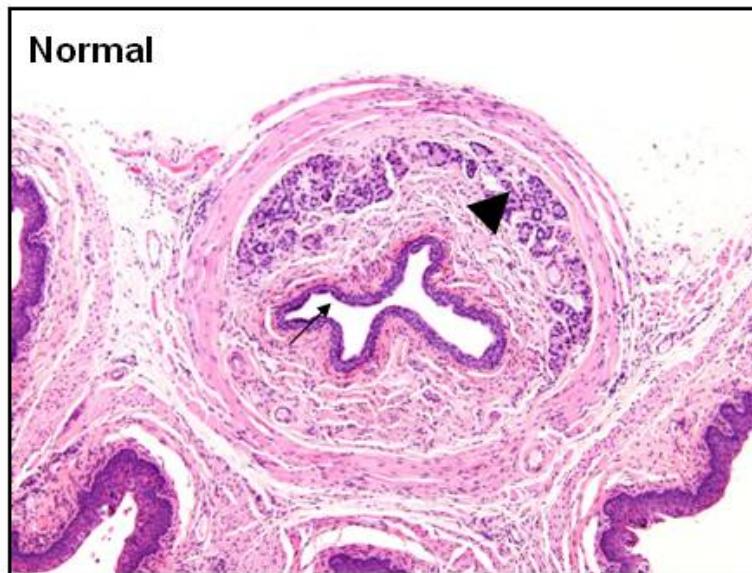
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## Neoplastic Lesions of the Urethra in B6C3F1 Mice

Transitional Epithelial Carcinoma	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
<b>Male</b>	0**	32**	46**	49**
<b>Day of first incidence</b>	-	380	358	206
<b>Female</b>	0*	0	0	2

N=49 or 50 animals; \* P≤ 0.05; \*\* P≤ 0.01





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## Neoplastic Lesions in B6C3F1 Mice

Male	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
<b>Lung</b>				
A/B Adenoma	5*	16**	12**	6*
A/B Adenoma or carcinoma	7*	17**	15**	6
<b>Forestomach</b>				
Squamous cell carcinoma	0	1	1	3*
Female	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
<b>Lung</b>				
A/B Carcinoma	0*	2	1	4*
A/B Adenoma or carcinoma	3*	8	5	10*
Cystic keratinizing epithelioma	0	0	0	2
<b>Forestomach</b>				
Squamous cell carcinoma	0	1	1	4*

N=49 or 50 animals; \* P≤ 0.05; \*\* P≤ 0.01



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## Neoplastic Lesions in Female B6C3F1 Mice

	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
<b>Skin</b>				
<b>Fibrosarcoma or malignant schwannoma</b>	2*	8	7	12**
<b>Malignant Lymphoma (HC mean 13%; range 4-22%)</b>	2	5	8*	7*

N=49 or 50 animals; \* P≤ 0.05; \*\* P≤ 0.01



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## Chloracne-Like Skin Lesions in B6C3F1 Mice

Male	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Hair follicle dilatation	7	10	13*	28**
Sebaceous gland atrophy	13	10	20**	29**
Female	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Hair follicle dilatation	2	0	11**	23**
Sebaceous gland atrophy	6	10	11	15*

N=49 or 50 animals; \* P≤ 0.05; \*\* P≤ 0.01



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## Inflammatory Skin Lesions in B6C3F1 Mice

Male	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Inflammation, chronic active	3	13**	12**	5*
Dermis				
Fibrosis	3	12**	12**	4
Epidermis				
Hyperplasia	3	13**	12**	6*
Ulcer	3	13**	11**	6*
Female	0 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Inflammation, chronic active	0	2	6*	11**
Dermis				
Fibrosis	0	1	4	11**
Epidermis				
Hyperplasia	1	2	4	11**
Ulcer	0	1	4	11**

N=49 or 50 animals; \* P≤ 0.05; \*\* P≤ 0.01



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## Conclusions in Male and Female B6C3F1 Mice

- *Clear evidence of carcinogenic activity* based on:
  - Transitional epithelial gland carcinoma of the urethra (Males)
  - Alveolar/bronchiolar neoplasms of the lung (Males)
  - Fibrosarcoma or malignant schwannoma (combined) of the skin (Females)
- Were also related to treatment:
  - Squamous cell carcinoma of the forestomach
  - Transitional epithelial gland carcinoma of the urethra (Females)
  - Alveolar/bronchiolar neoplasms of the lung (Females)
  - Cystic keratinizing epithelioma of the lung (Females)
- May have been related to treatment:
  - Malignant lymphoma (Females)



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## Comparison of Neoplastic Effects of TCAB and TCDD

- Target tissues in common
  - Adrenal gland
  - Liver
  - Lung
  - Oral Mucosa
  - Thyroid gland
- Target tissues observed with TCAB
  - Forestomach
  - Skin
  - Urethra
- Target tissues not observed with TCAB
  - Pancreas
  - Uterus